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14. ABSTRACT
This revised project has as its goal the characterization of a novel alternative product of the Her-2/neu/erbB2 protooncogene derived from intron retention. The product of this splicing mechanism, termed herstatin, is a secreted protein comprised of the N terminus of the Her-2 receptor tyrosine kinase and a unique, intron-encoded C terminus that allows binding to the other members of the EGFR/erbB family. This binding down-regulates erbB expression and inhibits EGF family signaling and cell proliferation. We have found that herstatin also binds to and down-regulates the IGF-I receptor (IGF-IR) and modulates IGF signal transduction. Herstatin is, therefore, a novel bifunctional inhibitor of erbB and IGF-IR signaling. Herstatin is expressed in prostate tissue and may represent a promising therapeutic target and biomarker in CaP. The work proposed in this project will assess the effect of herstatin on CaP cell phenotype, the expression of herstatin in CaP samples, and potential mechanisms of regulation of herstatin expression.

15. SUBJECT TERMS
IGF-I RECEPTOR, ERBB RECEPTORS, PROSTATE CANCER, METASTASIS

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Introduction

As described in the revised annual report submitted in 2006, we have re-oriented our effort on this project to elucidate the potential role of the novel Her-2-derived erbB autoinhibitor protein herstatin in CaP and its possible utility as a therapeutic target/diagnostic biomarker. Given the timing of the revised annual report, this report will describe the progress we have made in the latter half of the second year of funding (i.e., July-Dec 2006).

Body

In conjunction with the submission of the revised annual report for calendar year 2005, we proposed a modified Statement of Work to guide the research for the remainder of the project. This is shown below, with the relevant findings reported for each task for the period July-December 2006.

Task 1. Characterize the effects of herstatin in prostate cancer cells (months 7-12 of year 2 and 1-6 of year 3).

a. *Generate lentivirus constructs encoding herstatin and infect androgen-sensitive (LNCaP) and insensitive (PC-3) CaP cell lines.*
b. *Analyze IGF-I signaling in acutely and long-term infected cells.*
c. *Assess effects of herstatin on proliferation, migration, and apoptosis.*

We have constructed a lentivirus vector encoding herstatin and are currently generating active virus for transfection of AR-positive and negative cells.

Task 2. Evaluate expression of herstatin in prostate cancer cell lines and clinical samples (months 1-12 of year 3).

a. *Optimize quantitative RT-PCR amplicon probesets and determine herstatin vs HER2 mRNA levels in a series of CaP cell lines, including PC-3, PC-3/AR, LNCaP, DU-145, P69, M12, and 22rV.1.*
b. *Assay herstatin expression in normal prostate tissue and CaP biopsies (already obtained from OHSU Cancer Center tissue bank).*

We have designed and optimized herstatin-specific TaqMan amplicons for qRT-PCR analyses.

Task 3. Investigate regulation of herstatin expression (months 7-12 of year 2 and 1-12 of year 3).

a. *Based upon preliminary data suggesting that heregulin increases herstatin expression in SKOV-3 cells, we will determine the effects of EGF system ligands on herstatin mRNA levels in CaP cell lines that express endogenous herstatin.*
b. *Utilizing minigene constructs that allow the assessment of intron 8 retention, evaluate differential intron retention activity in CaP cell lines and the effect of EGF ligands on minigene activity.*
c. *Determine the effect of WT1 (+KTS) on herstatin expression and minigene activity. This experiment is based upon a recent report that a particular splice variant of the WT1 tumor suppressor (which we have previously shown to be present in CaP [1] regulates intron retention and the translation of mRNAs containing retained introns [2].*

In other studies, we have characterized the 3' end of the herstatin transcript and demonstrated that the polyadenylation site necessary for production of a functional herstatin mRNA is only present in primate DNA, suggesting that herstatin is a primate-specific gene product. This arrangement mirrors the characteristics of the retained intron 8 sequence that is responsible for the herstatin protein structure, in that primate intron-8 sequences encode an extended unique C-terminal 79 amino acid sequence, while rodent intron-8 sequences contain a proximal stop codon. As shown in Figure 1 on the following page, we have identified the 3' structure of the herstatin transcript as comprising Her-2 exon 8, intron 8, exon 9, and the 5' portion of intron 9. Thus, herstatin mRNA is the result of retention of both intron 8 as reported previously [3] and intron 9, which provides a consensus polyadenylation site embedded in a so-called TDE motif which has been recently reported to be necessary for efficient translation initiation of the Her-2 open reading frame [4]. Specifically, a sequence

similar to the TDE element described in the Her-2 3'-UTR (in exon 22) occurs in intron 9 of the human, rhesus, mouse, and rat Her-2 genes, but only contains a consensus polyadenylation site in the primate genes.

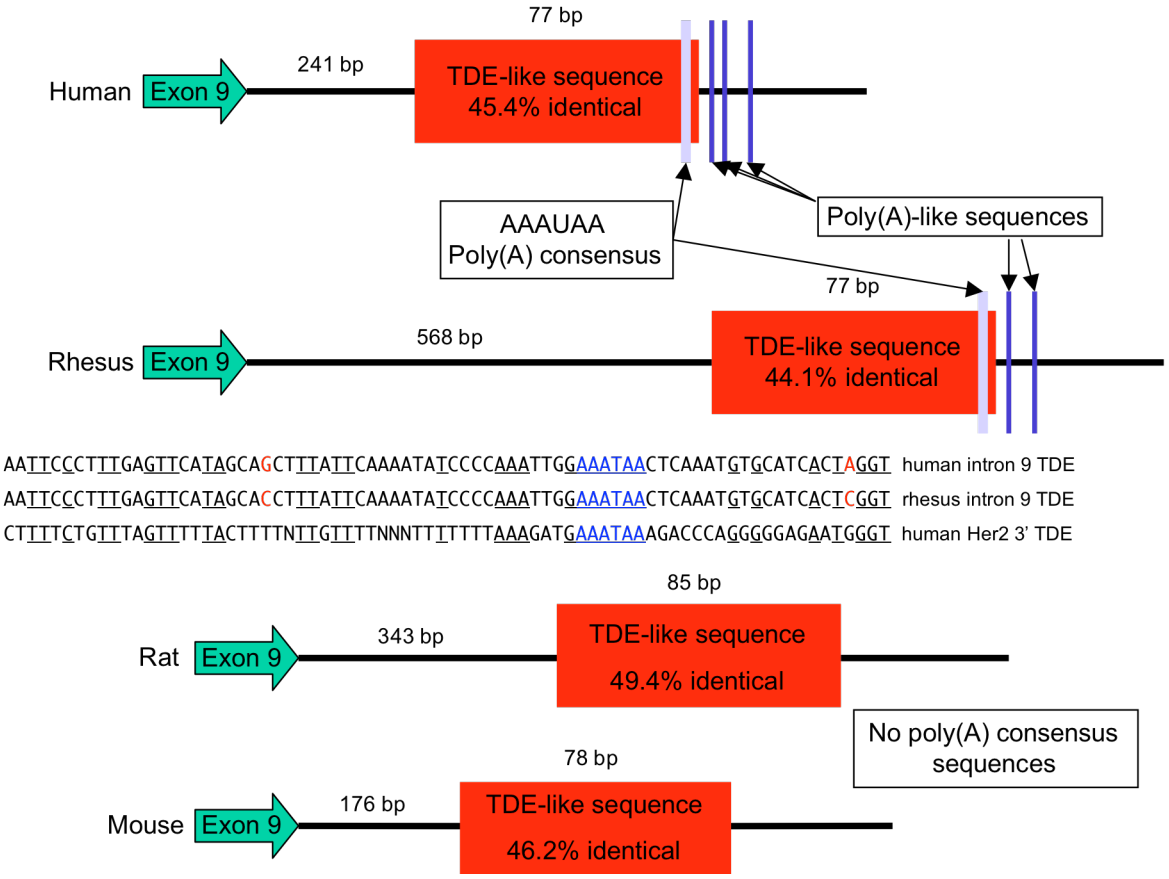

Figure 1. Location of TDE-like sequences in intron 9 of several mammalian Her-2 genes and conservation of sequence, including the consensus polyadenylation site, in primate intron 9 and human exon 23.

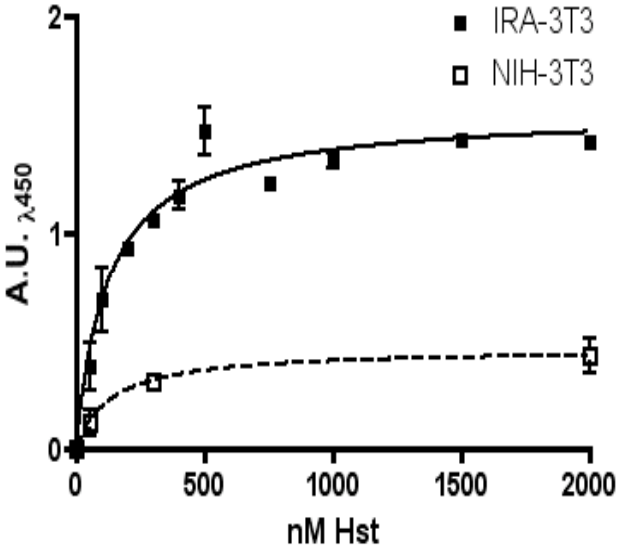

Figure 2. Cell-surface binding of recombinant herstatin to insulin receptor-expressing cells.

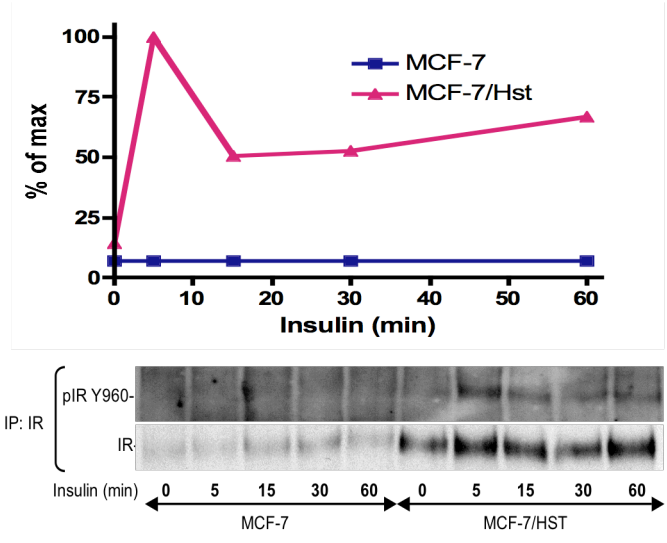

Figure 3. Stable expression of herstatin in MCF-7 cells increases insulin receptor expression and insulin-stimulated receptor activation

We have now also shown that herstatin binds to the human insulin receptor expressed in NIH 3T3 cells (Figure 2), but, unlike its effects on the IGF-IR and the EGFR, herstatin increases insulin receptor expression and activation (Figure 3). This finding is of particular importance to the potential of herstatin as an anti-cancer agent, since it demonstrates that it can inhibit EGFR family and IGF-IR expression and action without attendant undesirable inhibition of the insulin receptor, which would entail deleterious effects on glucose homeostasis.

Key research accomplishments

- Development of herstatin-specific qRT-PCR probes
- Preliminary mapping of herstatin transcriptional unit
- Demonstration of binding to insulin receptor
- Demonstration of enhancement of insulin receptor expression and activation

Reportable outcomes

None (3 manuscripts in preparation).

Conclusions

Our studies to date on herstatin demonstrate its ability to coordinately inhibit erbB and IGF-IR expression and signaling, with presumptive inhibitory effects on cancer cell proliferation and survival. Its utility as a possible therapeutic agent is enhanced by the demonstration of increased, rather than decreased, insulin receptor expression and action in cells expressing herstatin. Our characterization of the transcriptional structure of the herstatin mRNA will facilitate analyses of the regulation of herstatin expression.

References

1. Dong, G., Roopmathy, R., Vu, T., Hoffman, A. Rosenfeld, R., Roberts, C.T., Jr., Peehl, D.M., and Cohen, P. *J Clin Endocrinol Metab* (1997) **82**, 2198-2203.
2. Bor, Y., Swartz, J., Morrison, A., Rekosh, D., Ladomery, M., and Hammarskjöld, M.-L. (2006) *Genes & Dev* **20**, 1597-1608.
3. Doherty, J.K., Bond, C., Jardim, A., Adelman, J.P., and Clinton, G.M. (1999) *Proc Natl Acad Sci USA* **96**, 10869-10874.
4. Mehta, A., Trotta, C.R., and and Peltz, S.W. (2006) *Genes & Dev* **20**, 939-953.

Appendices

None.

Supporting data.

NA